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Magnetostrictive Particle Based Biosensors for in Situ and Real-Time Detection of Pathogens in Water

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ABSTRACT: Biosensors for in situ detection of pathogenic bacteria in liquid are developed using magnetostrictive particles (MSP) as the sensor platform. The sensing elements used are phage E2 against *Salmonella typhimurium*, monoclonal antibody against *Listeria monocytogenes*, polyclonal antibody against *Escherichia coli*, and polyclonal antibody against *Staphylococcus aureus*, respectively. These biosensors were characterized in cultures with different populations ranging from 5×10^1 to 5×10^8 cfu/mL. It is found that the MSP-based biosensors work well in water and have a rapid response with a response time in minutes, which makes the MSP-based sensors suitable for in situ and real-time detection of pathogenic bacteria in liquid. The experimental results show that all MSP-phage and MSP-antibody biosensors in size of 1.0 mm × 0.3 mm × 15 μm exhibit a detection limit better than 100 cfu/mL. Based on the Hill plot, it is concluded that each bacterial cell is bound onto the sensor surface through about four-to-five sites. When the cultures with low population ($<10^6$ cfu/mL) are tested, both MSP-phage and MSP-antibody sensors exhibit the similar response. However, the phage-MSP sensors exhibit a higher capability in the capture of target bacterial cell.

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KEYWORDS: biosensor; pathogen; phage; antibody; magnetostrictive particle

Introduction

Food safety is vital to everyone's health and represents a great threat to the public as evidenced by recent foodborne outbreaks of pathogen-related infections around the world. According centers for disease control and prevention (CDC), there are 9.4 million cases of foodborne illness each year in the United States alone (Scallan et al., 2011a,b). Among all foodborne illness about 45% are due to micro-organisms. Pathogenic bacteria are widely found in food, soil, water, and the intestinal tracts of humans and animals (Ivnitski et al., 1999). Some of them can withstand various harsh environments (Sams, 2001). Therefore, it is highly desirable to monitor/detect the presence of pathogens in food at an earlier stage to prevent food infection outbreaks. For the detection of pathogens in food, the food sample is usually prepared in liquid form by blending food with water. The infectious dose can be as low as 10–100 cells (e.g., *Escherichia coli*) (Bhunia, 2007). Therefore, a technology designed for the detection of pathogens in food should have two very critical features: works well in liquid so that the in situ detection can be performed and have a high sensitivity so that the presence of a small number of bacterial cells can be detected.

Many techniques, such as plate counting methods and enzyme-linked immunosorbent assay (ELISA), are currently widely used for the detection of microorganisms in liquid/food (El-Shaarawi and Piegorsch, 2002). These techniques require trained personnel and must be performed in a controlled environment such as a laboratory. Additionally, they have a low sensitivity when used as direct detection technique. The sensitivity of these techniques can be significantly improved if an incubation step is performed prior to the detection. However, the incubation process is time-consuming. In the last two decades, significant progresses have been made on the development of polymerase chain reaction (PCR). There are a variety of commercial PCR systems currently available on the market. PCR technology is often referred as the gold standard method. Due to the fact that only a small DNA segment of target species is used in PCR, there is a concern about its specificity. PCR technology is very sensitive in terms of the number of cells to be detected in one sample, however, the detection limit is only 500–1,000 cfu/mL in terms of the concentration. The lack of a rapid and sensitive detection method of

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foodborne pathogens is a principal impediment in identifying food contamination. Biosensors are widely considered as the next generation detection technology that meets the detection time and sensitivity requirements for pathogen detection in foods.

A typical biological/chemical sensor has three parts: a sensing element (i.e., biomolecular recognition element for biosensor); a transducer system (sensor platform); and a readout system (interrogation system) (Zourob et al., 2008). The reaction of sensing element with the target species results in physical/chemical changes in the sensing element and/or the environment. Therefore, the sensing element and its immobilization determine the specificity of the sensor. The change caused by the reaction between the sensing element and target species is measured using a transducer that is interrogated using a readout system. The sensitivity and response time of a sensor are determined by the sensing element and the transducer used in the sensor. The transducer is the key to determine whether a sensor can be used in liquid for in situ detection.

For the detection of pathogenic bacteria, there are many types of sensing elements, among them antibodies are widely used due to the fact that antibodies have a very high specificity. However, antibodies are usually both fragile and expensive and can only work within a narrow temperature range in a humid environment. In recent years, bacterial phage has been used as an alternative to antibodies. Phages can be produced in a laboratory and can perform within a broader temperature and humidity ranges than antibodies (Petrenko and Vodyanoy, 2003). However, the availability of phages is limited and there is a concern about its specificity since the growth technique is less mature compared with those for antibodies.

In order to develop high-performance biosensors, various types of transducer have been investigated, such as acoustic-wave (AW), electrochemical, electronic/electrical, and optical-based transducers. Each type has its own advantages and disadvantages, which are dependent on the targets. The electrochemical transducer is generally used in the situation where the reaction between the target species and the sensing element involves a charge/electron transfer (Pohanka and Skládal, 2008). The optical transducer is, in general, based on the change in optical properties over an area, which makes the optical transducers have great advantages in the detection of small molecules (Fan et al., 2008). An AW transducer is actually a mass sensor. Therefore, AW sensors are more suitable for the detection of particle-like bacterial cells. A cell has a mass of approximately 10^{-12} g. Therefore, a great deal of attention has been attracted on the development of AW-based biosensors for food safety application (Guilherme et al., 2009; Vo-Dinh and Cullum, 2000).

An AW sensor is actually a mechanical resonator, whose resonant frequency is used as the signal since the resonant frequency is directly related to the mass load attached onto the surface. Therefore, an AW sensor is usually characterized using two critical parameters—mass sensitivity (S_m) and quality merit factor (Q value) (Ballantine and White, 1997; Kao et al., 2009; Ilic et al., 2005; Yang et al., 2006). The S_m is defined as the change in its resonant frequency due to the attachment of a unit mass, while Q value reflects the sharpness of its resonance peak. A higher Q value means a sharper resonance peak that results in a higher resolution in the determination of the resonant frequency. Therefore, an AW sensor with a higher S_m and a higher Q value is highly desirable. In general, the smaller AW sensor is, the higher is S_m.

Various AW sensors have been investigated. For example, bulk AW sensors, such as quartz crystal microbalance (QCM) and surface acoustic wave (SAW), have been widely studied as transducers for the development of biosensors (Ballantine and White, 1997). Bulk AW sensors can reach a high sensitivity in terms of the thickness of a uniform film (Ballantine and White, 1997). For example, a 5-MHz QCM has a capability to detect a change in the thickness as small as 0.1 nm for a uniform film. However, in order to keep an AW sensor work properly, the active area of these bulk devices (i.e., vibration area or sensing area) cannot be too small so that these devices have some disadvantages in the detection in terms of mass. For example, the 5-MHz QCM requires an active area of at least 5 mm in diameter, which results in a minimum detectable mass of about 10^{-9} g—approximately 1,000 times higher than the mass of a bacterial cell. In order to increase the sensitivity, a great deal of research effort has been focused on the development of AW sensors with a smaller size/mass, such as MEMS/NEMS (micro/nano-electro-mechanical system). For example, a smaller QCM (Kao et al., 2009), micro/nano-cantilevers (Ilic et al., 2005), micro-diaphragm (Feng and Li, 2013; Lu et al., 2012; Yang et al., 2006), and other micro-resonators have been investigated as transducers used in the development of high performance biosensors.

Among these miniaturized AW sensors, the research on the micro/nano-cantilevers has achieved the most progress. Different micro/nano-cantilevers, such as silicon (Gupta et al., 2004; Ilic et al., 2001, 2005), piezoelectric (Capobianco et al., 2006; McGovern et al., 2007, 2008), and magnetostrictive (Fu et al., 2007; Li et al., 2006; Zhang et al., 2013) based cantilevers, have been investigated. The micro/nano-cantilevers exhibit an unprecedented S_m. For example, detections of a single *E. coli* cell and/or a mass of 10^{-18} g have been demonstrated (Gupta et al., 2004; Ilic et al., 2001) using silicon-based micro-cantilevers. However, these demonstrations were conducted in vacuum to avoid the low Q value associated with these cantilevers when they are operated in air/liquid due to the fact that the Q value is about 100 in air and less than 10 in liquid (Li et al., 2006). To use these AW sensors in liquid, various approaches have been introduced and investigated to improve their Q value. For example, a microfluidic system was integrated with a cantilever so that the cantilever is operated in vacuum with the liquid analyte to be tested in the cantilever (Burg et al., 2007). Cantilevers made of magnetostrictive materials were introduced as a type of cantilever with a higher Q value (i.e., more 30 in liquid) (Li et al., 2006), which make the magnetostrictive cantilevers

good for in situ detection of bacterial in liquid. The in situ detection capability of the magnetostrictive cantilever has been demonstrated by direct detection of microorganisms, such as *Salmonella typhimurium* (Fu et al., 2007), *E. coli* (Fu et al., 2010), and *Bacillus anthracis* spores in water (Fu et al., 2011).

By using magnetostrictive materials, other types of resonators have been developed and investigated as sensor platforms (Cheng, 2005; Grimes et al., 2011; Horikawa et al., 2011). Among these magnetostrictive resonators, magnetostrictive particle (MSP) is a type of transducer sensor platform that works well in liquid, exhibits a high sensitivity, and overcomes some intrinsic drawbacks of AW sensor platforms (Cheng, 2005; Li and Cheng, 2010; Zhang et al., 2013). For example, the Q value of an MSP in liquid can reach more than 100 (Cheng et al., 2008). The MSPs have been investigated as a transducer and the phage-based MSP biosensors have been developed (Wan et al., 2007).

In this article, the MSP biosensors using antibody as sensing element are developed using MSPs in size of 1 mm × 300 μm × 15 μm. The performance of MSP-antibody biosensor is compared with the MSP-phage biosensors by the in situ detection of different bacteria in water. All biosensors exhibited a detection limit better than 100 cfu/mL.

Principle of a MSP Biosensor

A magnetostrictive material is a material whose shape changes with magnetic field applied on. By using magnetostrictive materials, various sensors, actuators, and transducers have been developed (Liu et al., 2005). The principal advantage of magnetostrictive-based resonators over other resonators is that magnetostrictive resonators are wireless. That is, a magnetostrictive resonator is actuated/drove using a magnetic field and its oscillation/vibration behavior is sensed using a magnetic signal. A wireless resonator (wireless sensor platform) means that there is no physical connection between the sensor platform and the interrogation unit (measurement device), which is very interesting for the development of biosensors for in situ and in-field detections.

If a strip-like magnetostrictive material, magnetostrictive particle (MSP), is used as a magnetostrictive resonator, its resonant frequency for the vibration/oscillation along its length direction (longitudinal mode) can be expressed as (Li and Cheng, 2010):

$$f_{r,n} = \frac{n}{2L} v \quad n = 1, 2, 3\ldots \tag{1}$$

where $f_{r,n}$ is the resonant frequency of nth harmonic mode, L is the length, and v is the acoustic velocity of the materials, which is dependent on the elastic properties and the density of the material as well as the shape of the particle (i.e., the ratio of length to width) (Li and Cheng, 2010; Liang et al., 2007).

The S_m introduced in the introduction is defined as (Li and Cheng, 2010):

$$S_m = -\frac{\Delta f_n}{\Delta m} \tag{2}$$

where Δf_n is the change in the resonant frequency of nth harmonic mode due to the attachment of the mass load Δm onto the surface of the MSP. The S_m of an MSP can be further written as (Cheng, 2005):

$$S_m = \frac{f_{r,n}}{2M} \quad (\Delta m \ll M) \tag{3}$$

where M is the mass of the MSP without mass load and $f_{r,n}$ is defined in Equation (1). Based on Equations (1) and (3), one can find that the smaller the size of the MSP, the higher the S_m.

Materials and Methods

MSP Fabrication

Metglas™ 2826 MB ribbon (Honeywell International, Conway, SC), a commercially available magnetostrictive alloy, was used to fabricate the MSPs used in this study as described below. First of all, 2400# sand paper was used to publish the Metglas™ 2826 MB down to a thickness of 15 μm. Then, the polished Metglas™ 2826 MB ribbon was cut into rectangular particles in the size of 1.0 mm × 0.3 mm using a micro-dicing saw Model 1110 (MicroAutomation, Inc., Centreville, VA). These diced rectangular particles (i.e., magnetostrictive particles, MSPs) were cleaned in methanol for 30 min using an ultrasonic cleaner. These MSPs were immediately dried using nitrogen gas. The surfaces of the cleaned and dried MSPs were then coated with a bilayer—a layer of chromium (Cr) in thickness of 100 nm first and a layer of gold (Au) in thickness of 125 nm second—using a RF sputtering system (Denton Vacuum, LLC, Moorestown, NJ). The chromium layer was used to enhance the adhesion between the gold layer and the MSP, while the gold layer was used to promote the immobilization of sensing elements (i.e., phage and antibody in this study) and also prevent the MSPs from corrosion. The MSPs coated with Cr/Au were immediately used for immobilization of sensing elements.

Phage and Antibody Immobilization

In this study, both phages and antibodies were used as sensing elements. Each has some advantages and disadvantages as described in the introduction. The phage used is Phage E2 against *S. typhimurium* (Petrenko and Vodyanoy, 2003), while antibodies used were polyclonal antibody (PAbs) anti-*E. coli* rabbit IgG specific to *E. coli* from GeneTex, Inc. (Irvine, CA) (GTX13626), monoclonal antibodies (MAbs) anti-*Listeria monocytogenes* rabbit IgG specific to *L. monocytogenes* from

Purdue University, and polyclonal antibody anti-*Staphylococcus aureus* IgG specific to *S. aureus* from 3M, respectively.

Regarding the immobilization process, phages can be immobilized onto gold surface using different techniques/processes depending on the type of phage. Phages can have different sizes/lengths. A lytic phage, such as T4 bacteriophage, is only about 200 nm long with a head that is about 60 nm in diameter (Gervais et al., 2007). The lytic phages can be covalently immobilized onto gold surface with a desired orientation (Tolba et al., 2010), which certainly enhance the performance of the biosensor. The phage used in this work is filamentous phage, which has a length of 800–900 nm and a diameter of 6–7 nm (Petrenko, 2008; Petrenko and Vodyanoy, 2003). Our previous research has demonstrated that, using physical absorption, this kind of phage can be immobilized onto a gold surface with high density and good stability. In this work, the phage was immobilized onto gold-coated MSP surface using physical absorption. That is, a phage suspension was first prepared at room temperature with a population of 1.05×10^{12} virions/mL. The phage suspension was poured into containers, each with a volume of 200 μL. An MSP coated with Cr/Au was immersed into the phage suspension in one of the containers for 1 h at room temperature. During the immobilization, the container with the MSP and phage suspension was continuously rotated using a Labquake Tube Shaker/Rotator (Barnstead/Thermolyne Corp., Dubuque, IA). After that, the MSP was taken out and rinsed with sterile distilled water for three times to remove the unimmobilized phages. Now, an MSP is an MSP-phage biosensor.

Regarding antibody immobilization, physical adsorption was used first. However, it was experimentally found that the physical adsorption does not result in a high quality of antibody immobilization. Then, a methodology to immobilize the antibody onto the MSP surfaces through covalent bonds was developed. The details of the immobilization can be found in Fu's dissertation (Fu, 2010). In brief, Traut's Reagent was used to modify the antibody by introducing the sulfhydryls into the antibody, which can be covalent bond with gold. The modified antibody was then attached to MSP coated with Cr/Au through a covalent bond.

Experimental Setup

The characterization of the biosensors was carried out using a home-made setup as shown in Figure 1, where two reservoirs are connected with a tube. The MSP biosensors were placed in the tube. A peristaltic pump was used to force the sample to be tested flowing in the tube. Home-made coils were wound on the outside of the tuber to interrogate the MSP biosensor in the tube using a network analyzer (HP 8751A with HP 87511) that is operated using *S*-parameters. A magnet was placed on the outside of the tube to provide a dc magnetic field.

The measured S_{11} signal includes both amplitude and phase. A typical set of results is given in Figure 2, in which the frequency range is from 2.25 MHz to 2.27 MHz for an MSP with a length of 1 mm. As shown in Figure 2, there are three characteristic frequencies (i.e., f_r, f_0, f_{ar}). These three frequencies reflect the resonant behavior of an MSP biosensor. In this study, the characteristic frequency f_0, at which the amplitude signal reaches its minimum, was used as the output signal of the MSP biosensor.

The f_0 obtained at different time is plotted as a function of time to determine the performance of an MSP biosensor as described below. The reproducibility of the f_0 obtained from S_{11} signal is dependent on two factors: the measurement error of the network analyzer and the stability of the resonant frequency of the MSP sensors. For the network analyzer used here, the error in the S_{11} measurement is much smaller than the stability of the MSP resonator. The stability of the MSP resonator was tested in air and water at room temperature.

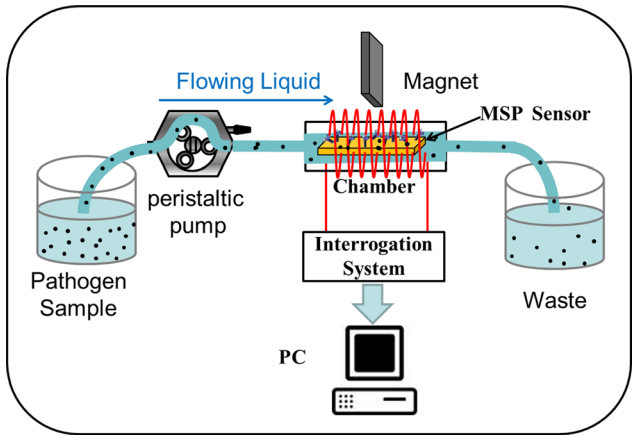

Figure 1. Schematic experimental setup for the characterization of the response of the MSP biosensor in liquid analyte, where the interrogation system is a network analyzer.

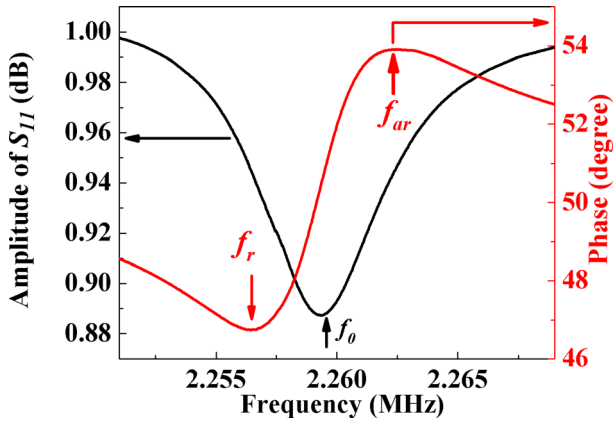

Figure 2. A typical set of results obtained in one measurement: frequency dependence of amplitude and phase of S_{11} signal from an MSP in size of 1.0 mm × 0.3 mm × 15 μm in air.

For the MSPs used in this study, the experimental results indicate that the resonant frequency changes with time with a standard deviation less than 40 Hz when the MSP is operated in water.

Experimental Procedure

In the experiment, an MSP biosensor was first placed into the test chamber (tube) and then the peristaltic pump was set with a flow rate of 30 μL/min. During measurement, sterile distilled water was used first. Then, the cultures with different populations were used one by one as the population was increased. The populations used were 5×10^1, 5×10^2, 5×10^3, 5×10^4, 5×10^5, 5×10^6, 5×10^7, and 5×10^8 cfu/mL, respectively. The sample (i.e. water and culture) was driven to flow through the tube until the sensor reached its stable response. The test duration for each sample was approximately 20 min. The response (i.e., characteristic frequency f_0) of the sensor in the water was used as a background. Then, the response of the sensor in each culture was tested and recorded using a computer with an interval of 30 s.

After the final test using culture with the highest population (5×10^8 cfu/mL), the MSP biosensor was rinsed using deionized (DI) water three times and, then, dried in air. Then, scanning electron microscopy (SEM) was used to observe the sensor surface to verify whether the change in the characteristic frequency of the MSP biosensor was due to the binding of target bacterial cells. Prior to SEM observation, the sensor was exposed to osmium tetroxide (OsO_4) vapor at room temperature for 1 h. Osmium tetroxide is used to keep the initial shape of the bacterial cells in high vacuum during SEM experiment. The SEM observation was performed on a field emission scanning electron microscope JEOL 7,000 F at 20 keV.

Results and Discussion

The data shown in Figure 3 is the change in the characteristic frequency with time for an MSP-phage biosensor in *S. typhimurium* cultures with different populations. The results shown in Figure 3 represent a typical set of experimental results (dynamical response), where the characteristic frequency f_0 of the MSP biosensor is plotted as a function of time. As shown in Figure 3, the test was done first using water. When a *S. typhimurium* culture is brought into the test chamber (tube), the f_0 drops and eventually reaches its saturated value. Then, when a *S. typhimurium* culture with a higher population is brought into the test chamber, the f_0 drops again and eventually reaches a new saturated value. From the data shown in Figure 3, one can find that the MSP-phage biosensor has a rapid response with a response time in minutes, which makes the MSP-phage biosensors good for real-time detection.

Based on the dynamic response curve shown in Figure 3, a so-called dose–response curve is generated as shown in Figure 4a, where the frequency shift is the saturated f_0 of the

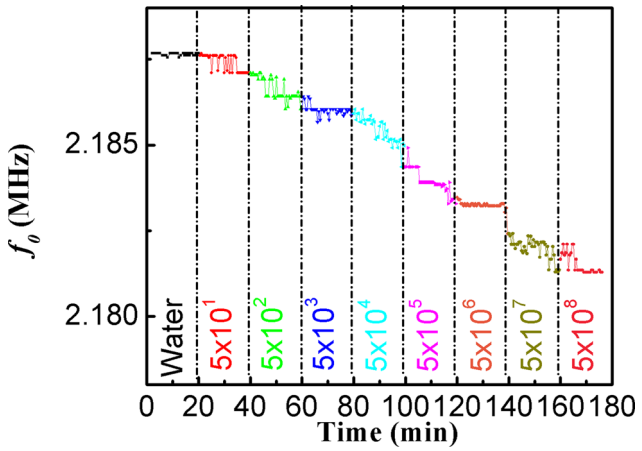

Figure 3. A typical dynamic response of an MSP biosensor: the characteristic frequency f_0 of an MSP biosensor versus time. Here, the size of the MSP is 1.0 mm × 0.3 mm × 15 μm. The phage immobilized onto the MSP surface is phage E2 against *S. typhimurium*. The population of *S. typhimurium* culture used in the experiment at different times is given in the figure in unit of cfu/mL.

MSP sensor in each culture minus the stable f_0 of the MSP sensor in water. From the data, it is found that a linear dose response was observed in a population range from 5×10^3 to 5×10^7 cfu/mL, which results in a sensitivity (the slope of the linear range on the dose–response curve) of about 1,000 Hz per decade ($R^2 = 0.989$) for the sensor. The solid curve in the figure is the fitting results using Sigmoidal function (Fu et al., 2010, 2011):

$$Y = A_2 + \frac{A_1 - A_2}{1 + (X/X_0)^p} \tag{4}$$

where A_1, A_2, X_0, and p are fitting constants, X and Y (i.e., the x-axis and y-axis of the figure) are the population of the culture and the frequency shift of the sensor in each culture, respectively.

The dose–response curve shown in Figure 4a presents the performance of the biosensor. From a dose–response curve, one can determine the minimum detection limit of the biosensor. For the sensors studied here, a minimum detection limit less than 100 cfu/mL was obtained. Based on the dose–response curve, the Hill plot for the sensor can be generated (Fu et al., 2011). That is, the ratio of the frequency shift (Δf, y-axis) in Figure 4a for each population to the fitted "A_2" in Equation (4) is defined as $Z(=\Delta f/A_2)$. Then, the Hill plot for the sensor is given in Figure 4b, where the y-axis is the logarithmic scale of "Z/(1 − Z)". A Hill coefficient (the slope of the fitted solid line) of 0.2578 was obtained. This indicates that one *S. typhimurium* cell was bound onto the sensor surface through about four binding sites.

It is found that the frequency shift for the sensor in the culture with a population of 5×10^1 cfu/mL is about 500 Hz, which is significantly higher than the standard derivation

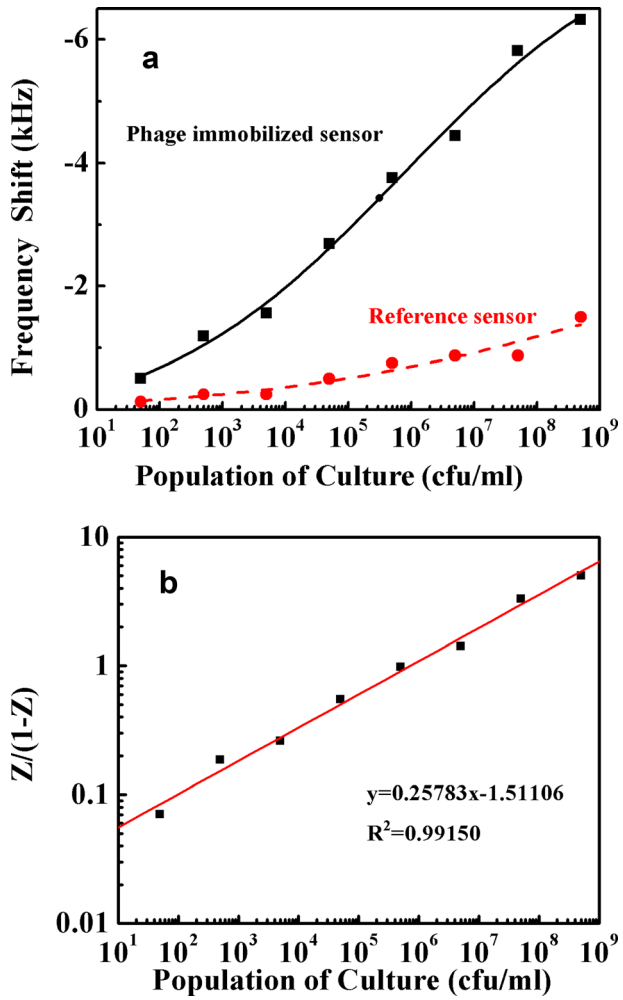

Figure 4. (**a**) Dose Dose–response curve (i.e., frequency shift vs. population of the culture) and (**b**) Hill plot of the MSP-phage sensors for the detection of *S. typhimurium* in water. Here the results from MSP biosensors (i.e., sensor) and a reference MSP sensor (i.e., reference sensor) are presented. Both the sensors and reference sensor have a size of 1.0 mm × 0.3 mm × 15 μm. The MSP sensor is the MSP coated with E2 phage against *S. typhimurium*, while the reference sensor is an MSP without sensing element.

obtained for the sensor in water. That is, the MSP biosensor used here can identify the culture with a population as low as 5×10^1 cfu/mL, which is much lower than the detection limits (mostly 10^3 cfu/mL) reported for other sensors. As mentioned, the sensitivity of MSP sensor increases with reducing size. Therefore, it is highly expected that an MSP sensor with a smaller size would exhibit a better performance or can identify the culture with a much lower population.

In the performance characterization of a biosensor, it is very critical to confirm the sensor response is indeed due to the reaction between the sensing element and target species. This was done in this study using SEM observation. Another important issue is the non-specific binding. In this study, the non-specific binding of the MSP biosensor was studied using a reference sensor that is an MSP with the same size as the MSP biosensor but without sensing element immobilized onto its surface. The dynamical response curve of a reference sensor was measured first and then the dose–response curve of the reference sensor is determined. The difference in the dose–response curve between an MSP biosensor and a reference sensor presents the performance of the biosensor. As shown in Figure 4a, for the MSP-phage biosensors developed here for the detection of *S. typhimurium*, the non-specific binding is very small as evidenced by a very small frequency shift observed from the reference sensor. For example, for the sensors in the culture with a population of 5×10^1 cfu/mL, the frequency shift of the reference sensor is less than 130 Hz, which is much smaller than the 500 Hz obtained from the MSP sensor. The SEM pictures shown in Figure 5 were taken from MSP biosensor and reference sensor, respectively, after they were exposed into *S. typhimurium* cultures with the sequence and population shown in the top of Figure 3. The SEM results confirm that the observed frequency shift is due to the capture of target bacterial cells. That is, the sensor response is indeed due to the capture of target bacterial cells in the analyte.

For MSP biosensors using antibodies as sensing element, three kinds of biosensors were developed for the detection of *E. coli*, *L. monocytogenes*, and *S. aureus*, respectively. These biosensors were tested in *E. coli*, *L. monocytogenes*, *S. aureus* cultures, respectively, using the population sequence shown in the top of Figure 3. Based on the dynamical responses of these biosensors, the dose–response curves of these MSP-antibody biosensors were determined.

The data shown in Figure 6a is the results obtained from MSP-antibody sensors for the detection of *E. coli* in water. The results shown in Figure 6a are similar with the results shown in Figure 4a. Again, a linear dose response is observed over a population range from 5×10^2 to 5×10^7 cfu/mL, which results in a sensitivity of about 700 Hz per decade ($R^2 = 0.992$). It is found that the sensors in the sample with a population of 5×10^1 cfu/mL exhibits a frequency shift of about 250 Hz, which is well above the standard derivation (<40 Hz) observed for the MSP sensors in water and is also well above the frequency shift observed in the reference sensor. That is, the detection limits of the MSP-antibody sensors in size of 1.0 mm × 0.3 mm × 15 μm is as low as 5×10^1 cfu/mL, which is similar to the MSP-phage sensors. In the experiments, it was also found that these sensors also have a rapid response with a response time in minutes. That is, the MSP-antibody biosensors are also good for real-time detection. The SEM observations (not shown here) indicate that the observed shift in the characteristic frequency of the MSP-antibody sensors is indeed due to the capture of target bacterial cells onto the surface of the MSP sensors.

The Hill plot of the MSP-antibody sensors for the detection of *E. coli* in water is presented in Figure 6b. It is found that the sensors have a Hill coefficient of 0.2783. That is, one *E. coli* cell was bound onto the sensor through about four sites.

For the detection of *L. monocytogenes* in water and the detection of *S. aureus* in water, the similar results are observed as shown in Figure 7a. It is found that the linear dose range is

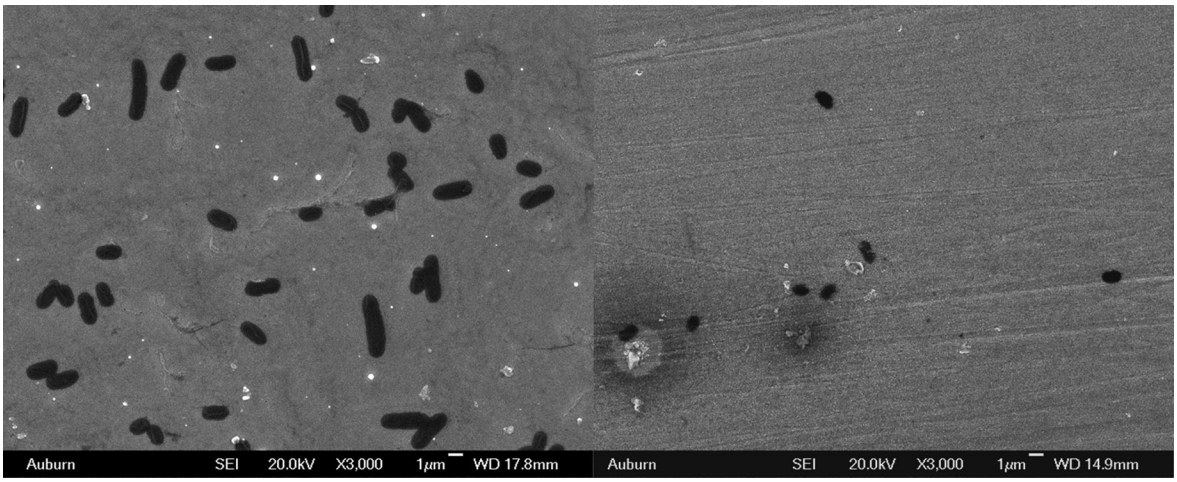

Figure 5. SEM observation of bacterial cells bond on the surface of sensors. **a**: MSP biosensor using phage E2 against *S. typhimurium* as sensing element. **b**: Reference sensor—an MSP without phage. Both MSP biosensor and reference sensor were exposed into *S. typhimurium* cultures with the sequence shown in top of Figure 3.

from 5×10^3 to 5×10^7 cfu/mL for the detection of *L. monocytogenes* using MSP-antibody sensors with a sensitivity of 600 Hz per decade ($R^2 = 0.990$), while the linear dose range is from 5×10^3 to 5×10^7 cfu/mL with a sensitivity of 500 Hz ($R^2 = 0.998$) for the detection of *S. aureus* using MSP-antibody sensors. Comparing the data shown in Figure 7a with the results shown in Figures 4a and 6a, one can find that all the MSP-antibody sensors and the MSP-phage sensors exhibit a similar sensitivity and the linear dose range is also very close.

For the MSP-antibody biosensors in the sample with a population of 5×10^1 cfu/mL, the frequency shift of the sensors is about 250 Hz for *E. coli* detection, 750 Hz for *L. monocytogenes* detection, and 750 Hz for *S. aureus* detection. These observed frequency shift is well above the standard derivation (<40 Hz) observed for the MSP sensors in water. That is, the detection limits of an MSP-antibody biosensor in size of 1.0 mm × 0.3 mm × 15 μm is much less than 100 cfu/mL, which is similar to the MSP-phage sensors. Again, similar with the MSP-antibody sensors for the detection of *E. coli*, the MSP-antibody sensors for detections of *L. monocytogenes* and *S. aureus* also exhibited a rapid response (i.e., a response time in minutes) and it is confirmed using the SEM observations that the observed shift in the characteristic frequency of the MSP-antibody sensors is indeed due to the capture of target bacterial cells onto the surface of the MSP-antibody sensors.

The Hill plots for these MSP-antibody sensors are presented in Figure 7b. It is found that the sensors designed for *L. monocytogenes* detection have a Hill coefficient of 0.2087 (i.e., one *L. monocytogenes* cell was bound onto the sensor through about five binding sites), while the sensors designed for *S. aureus* detection have a Hill coefficient of 0.2714 (i.e., one *S. aureus* was bound onto the sensor through about four binding sites). Recall that MSP-phage sensors for *S. typhimurium* detection has a Hill coefficient of 0.2578 and the MSP-antibody sensors for *E. coli* detection have a Hill coefficient of 0.2783, it seems that there is no significant difference in the Hill coefficient between the MSP-phage and MSP-antibody sensors. Based on the Hill coefficient and the sensitivity for the linear dose range discussed above, one can find that the MSP-antibody biosensors are the similar performance as the MSP-phage sensors. Indeed, from the data shown in Figures 4a, 6a, and 7a, it is found the MSP-antibody sensors have a similar response as the MSP-phage sensors in cultures with a low population ($<10^6$ cfu/mL). This means that both MSP-antibody and MSP-phage biosensors have a similar performance initially.

It is interest to compare the performance of MSP-based sensors exposed in the cultures with a high population. It is found that the MSP-phage biosensors exhibit a higher shift in the characteristic frequency than the MSP-antibody biosensors. For example, after exposed the MSP-based sensors into 5×10^8 cfu/mL culture, the frequency shift reaches about 6.3 kHz for the MSP-phage sensors, while it is about 4.2–4.6 kHz for all three types of the MSP-antibody sensors. That is, although both MSP-antibody and MSP-phage biosensors have a similar performance initially, the MSP-phage biosensors have a higher capability in the capture of target bacterial cells than the MSP-antibody biosensors. This is due to the facts that a phage is like a thin string (length about 850 nm and diameter about 6.5 nm) (Petrenko, 2008) and most of immobilized phages are attached onto the MSP surface through one of two ends (Fu, 2010). Therefore, the MSP-phage biosensors have a high capability in the capture/binding of target bacterial cells.

Application Remarks

The results reported here demonstrate that MSP-based biosensors have a rapid response and work well in the liquid (i.e., water). Therefore, MSP-based sensors can be used for in

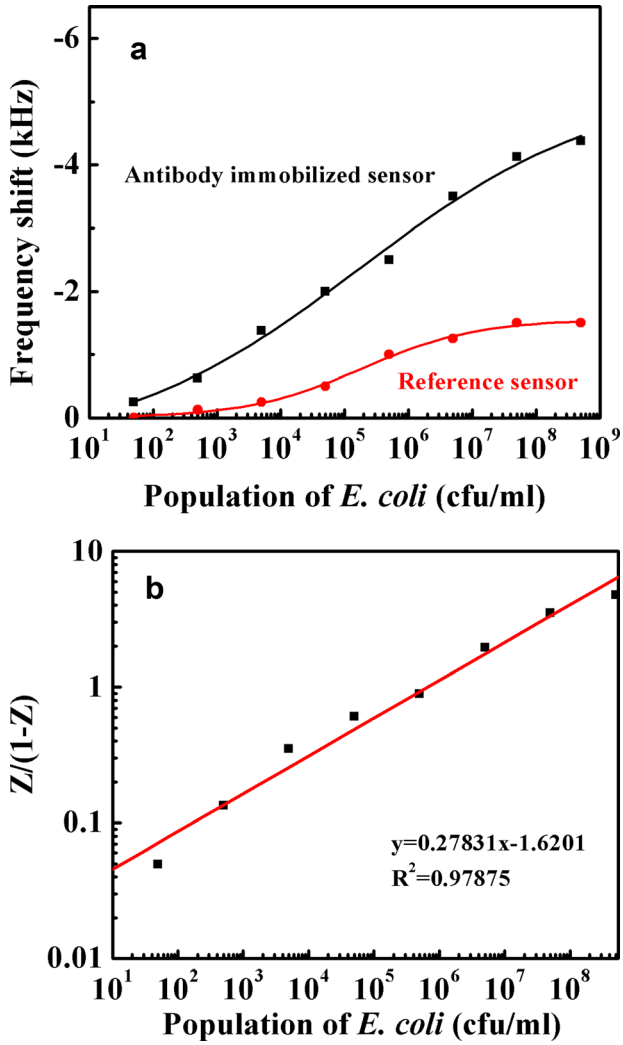

Figure 6. (**a**) Dose–response curve and (**b**) Hill plot of MSP-antibody biosensors for the detection of *E. coli* in water. The size of these MSP biosensors is 1.0 mm × 0.3 mm × 15 μm.

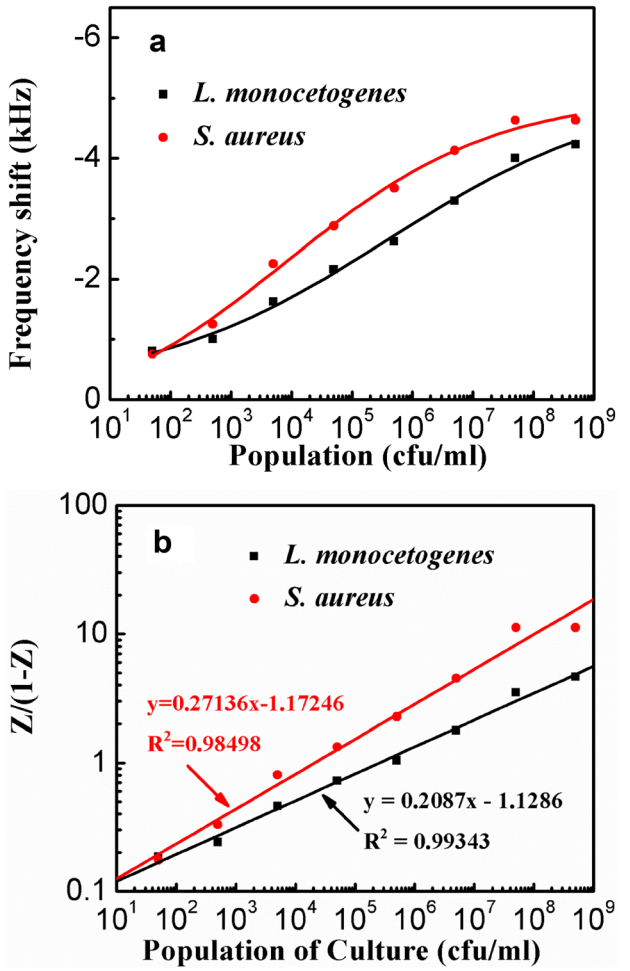

Figure 7. (**a**) Dose–response curves and (**b**) Hill plot of MSP-antibody biosensors for the detection of *L. monocytogenes* in water and *S. aureus* in water, respectively. The size of these MSP-antibody biosensors is 1.0 mm × 0.3 mm × 15 μm.

situ and real-time detection of bacteria. The results indicate that the sensitivity of the MSP-based sensors, with dimension of 1 mm × 300 μm × 15 μm, are better than 10^2 cfu/mL. Based on the principle of MSP sensor platforms, a higher sensitivity can be achieved by using MSPs with a smaller size.

Regarding applications, MSP-based sensors can be used to monitor/detect the presence of target bacteria in food and clinical samples as an in-field screening test technique. For food safety applications, a sample in liquid form is usually prepared first by blending food with water. For various test foods, the liquid samples prepared can be very different. For example, the liquid samples may have very different content of solid compounds, fiber, and oil/grease, which may affect the performance of the MSP-based sensors (i.e., resonant behavior of the MSP). It should be mentioned that the influence of these compounds on the sensor would result in a larger shift in the resonant frequency than without these compounds in the samples. That is, if these compounds have some influence on the sensors performance, the sensors will give a higher signal (i.e., false positive). This can be solved by separating the sensors from the liquid sample if a positive signal is obtained. This can be easily done since the MSP-based sensors, like the widely used magnetic beads (Li et al., 2003), can be collected using a magnet (Cheng, 2005; Zhang et al., 2013). Then, the sensors can be washed using water and analyzed in air. The MSP-based sensors actually have a better performance in air than in liquid since the *Q* value of the sensor in air is much higher than in the liquid (Cheng et al., 2008). This is an advantage of MSP-based biosensors over others. That is, if a positive signal is observed from the screening test using MSP-based sensors, a further analysis of the same sensors can be conducted.

For clinical diagnoses, body fluid such as urine and blood will be the samples to be tested. Based on communication

with researchers at 3M, a sensitivity of about 100 cells would be good enough for many clinical applications. The concerns for the clinical diagnosis are the background factors (i.e., pH value of the body fluid and other bacteria that may be present in the sample) within the body fluid. The influence of the pH value on the performance of the MSP-phage and MSP-antibody sensors has to be studied. The influence of other bacteria presented in the samples on the sensor performance is related to the specificity of the sensing elements, which can be solved by using a highly specific sensing element.

It is of interest to compare the MSP-based sensors with other detection technologies, especially the widely used flow cytometry, which is based on optical techniques. In flow cytometry, the information obtained is the scattered light from the target species. The scattered light only reflects the physical characteristics (i.e., color, size, and shape) of the target species. Therefore, specificity can be a great concern for flow cytometry. This concern for flow cytometry has been solved by using labeled antibodies (i.e., fluorescent dyes and even quantum dots conjugated to antibodies) that provide additional color information to the target species. In this way, the labeled antibodies have to be bounded with the target species in the sample. That is, a sample preparation process or sample pre-treatment (i.e., mixing labeled antibodies with sample and making sure the target species are bonded with the labeled antibodies) is needed and is critical to the performance of flow cytometry. Additionally, since the relationship between the scattered light and the physical characteristics is very complicated, a special analysis process of the experimental data has to be carried out using a computer to get the final results about the targets. However, for the MSP-based sensor approach reported here, there is no need to use the labeled antibodies or to pretreat the sample to be tested. That is, the sample to be tested is directly used in this MSP-based sensor approach. Additionally, there is no need to further analyze the data with flow cytometry since the results from the MSP-based sensors directly indicate the concentration of the target species. It should be mentioned that the bacterial detection limit with flow cytometry is about 10^3 cfu/mL (Gruden et al., 2004; Langerhuus et al., 2013). That is, the MSP-based sensor approach has a better detection limit.

From the pointview of real applications, cost is certainly an issue for any detection technology. For detection using the MSP-based sensors reported here, the cost can be categorized into the MSP-based sensors and the interrogation unit/system. The cost for fabricating MSP-based sensor is mainly the sensing element (i.e., phage or antibody), which is the same for all bio-detection technologies. Actually, the cost for antibodies used in the MSP approach is lower than other approaches that use the labeled antibodies since labeling antibodies means additional cost. Additionally, since the sensing element is only used to cover the surface of MSP sensors, the amount of antibody to be used in one analysis with the MSP-based approach would be less than that with other approaches which require the mixing of the antibody with target species in the sample to be tested. Regarding the interrogation unit/system, a network analyzer was used with some home-made coils as mentioned Experimental Setup Section. The cost of the coils (i.e., a few dollars) is ignorable compared with the network analyzer ($50k). However, as demonstrated by a new design developed in our lab (Cheng et al., 2012), a special circuit for interrogating MSP-based sensors can be prepared for lower than $500 per unit. For a measurement system, one would need this interrogation unit (<$500) and a laptop computer (~$2,000).

Conclusions

Magnetostrictive particle (MSP) as a sensor platform is used to develop high-performance biosensors for the detection of pathogenic bacteria in liquid. Both phages and antibodies are used as sensing elements. These biosensors were tested in bacterial cultures with different populations. First of all, it is found that all MSP biosensors have a rapid response with a response time in minutes. The in situ detection results indicate that the biosensors using MSPs in size of 1.0 mm $\times$ 0.3 mm $\times$ 15 μm have a detection limit better than 100 cfu/mL. Both MSP-phage and MSP-antibody sensors exhibit a similar response when the cultures with a population lower than 10^6 cfu/mL. However, the MSP-phage sensors exhibit a higher capability in the capture of bacterial cells than the MSP-antibody sensors, which results in a larger response in the MSP-phage sensors than the MSP-antibody sensors when the sensors are exposed in the cultures with high populations. Based on the Hill plot, it is found that each bacterial cell was bound to the sensor surface through four to five sites.

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